BACTERIAL PATHOGENESIS

Anthrax researchers complete triumvirate

DOI:
10.1038/nrmicro1416

URLs

Links:
Bacillus anthracis
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=12333

PA
http://ca.expasy.org/uniprot/P13423

EF
http://ca.expasy.org/uniprot/Q52NH2

LF
http://ca.expasy.org/uniprot/P15917

ATR
http://ca.expasy.org/uniprot/Q9H6X2

CMG2
http://ca.expasy.org/uniprot/P58335

LRP6
http://ca.expasy.org/uniprot/O75581

Bacillus anthracis produces two bipartite toxins, which have different cellular effects. Both contain the same receptor-binding moiety, protective antigen (PA), and this is combined with different effector moieties — PA in combination with oedema factor (EF) forms oedema toxin (ET), an adenylate cyclase that increases the intracellular concentration of cAMP, causing oedema, whereas PA in combination with lethal factor (LF) forms lethal toxin (LT), a metalloproteinase that cleaves mitogen-activated protein kinase kinases, disrupting intracellular signalling.

The mechanism of entry of the toxins into host target cells is relatively well understood, with the exception of one missing element. Two cell-surface proteins have been confirmed as receptors for PA, the anthrax toxin receptor (ATR) and capillary morphogenesis protein 2 (CMG2), but it has long been suspected that a third cell-surface protein might be involved in internalization of the toxin–receptor complex, as PA binding to ATR or CMG2 alone is not sufficient for toxin entry. The third protein has now been identified by Wensheng Wei and Quan Lu in Stanley Cohen's lab, in collaboration with G. Jilani Chaudry and Stephen Leppla, and is reported in a recent issue of *Cell*.

The authors set out to try and identify cellular genes involved in LT/ET toxicity using a phenotypic screen in which a lentivirus vector was used to introduce antisense RNAs against chromosomal genes into a human cell line, and clones deficient in PA internalization could be selected. Screening of the selected clones led to the identification of the gene encoding low-density lipoprotein receptor-related protein 6 (LRP6), a cell-surface protein known to be a co-receptor for Wnt signalling.

The observation that murine macrophages transfected with a lentivirus vector expressing *lrp6*-specific short interfering RNAs showed improved survival when challenged with native LT compared with cells transfected with control vectors, coupled with western blot and deconvolution microscopy analyses estimating the abundance of cell-surface and internalized PA in the absence of LRP6, suggested that LRP6 might be involved in PA internalization. This was confirmed in further work using a dominant-negative LRP6 mutant protein lacking most of the cytoplasmic domain, which also proved not only that LRP6 is necessary for toxin lethality and not just internalization, but also that the intracellular domain of LRP6 is required for both functions. To establish whether LRP6 interacts with either ATR or CMG2 and if so, what the functional significance of this interaction is, co-immunoprecipitation and fluorescence microscopy were used, and it was found that LRP6 interacts with both ATR and CMG2, and the authors propose a model in which this interaction provides the signal for internalization of the toxin–receptor complex.

The paper concludes with data showing that anti-LPR6 antibodies can protect a macrophage cell line from the lethal effects of LT, suggesting that this work could have ramifications for the treatment of the later stages of anthrax disease.

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ORIGINAL RESEARCH PAPER Wei, W. *et al.* The LDL receptor-related protein LRP6 mediates internalization and lethality of anthrax toxin. *Cell* **124**, 1141–1154 (2006)